

雷公1号柚果实无核成因探究

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摘要:【目的】探究雷公1号柚果实无核成因, 为无核果实稳定生产与无核育种提供理论依据。【方法】以雷公1号柚为母本, 通过对其花形态结构观察、花粉活力测定、自交授粉以及与鸡尾葡萄柚、春香橘柚和三红蜜柚为父本开展杂交试验, 分析雷公1号柚无核成因。【结果】雷公1号柚花器官结构完整, 且花粉活力较高, 表明雄性可育。该品种去雄不授粉的坐果率为14%, 果实均无核, 具备一定的单性结实能力。雷公1号柚自交授粉后, 花粉管在花柱中生长受阻, 自交坐果率12.00%, 单果种子数0.92粒·果⁻¹, 产生的11粒种子均为干瘪种子, 表现为自交不亲和; 但以其为母本与鸡尾葡萄柚、春香橘柚和三红蜜柚为父本进行有性杂交, 父本花粉管能够生长至花柱底端, 产生的种子多为饱满种子, 表现为异交亲和。雷公1号柚*S-RNase*基因型为 S_3S_3 , S_3 与 S_3 均在雌蕊组织中特异性表达, 且均在花柱中相对表达水平最高。【结论】雷公1号柚无核果实的形成由自交不亲和与单性结实共同导致, 且该品种自交不亲和类型属于*S-RNase*介导的配子体型自交不亲和。

关键词: 雷公1号柚; 无核; 自交不亲和; *S-RNase*

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Mechanisms underlying the formation of seedlessness in Leigong No. 1 pummelo

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Abstract: 【Objective】Seedlessness is a highly desirable horticultural trait in citrus and has emerged as a major breeding target due to consumer preference for seedless fruit. Multiple mechanisms can generate seedless citrus, including male sterility, female sterility, embryo abortion, and self-incompatibility. For pummelo cultivars, self-incompatibility is the predominant cause of seedlessness. Recent studies on several local pummelo varieties, such as Juxianghong pummelo, Dongshi Zaoyou pummelo, and Gongshui Baiyou pummelo, have demonstrated that their seedlessness primarily results from self-incompatibility. Citrus self-incompatibility has been demonstrated to be a gametophytic system mediated by *S-RNase*, whereby pollination between identical *S*-genotypes fails to produce normal seeds, whereas cross-pollination between different *S*-genotypes yields viable seeds. The combination of self-incompatibility and parthenocarpic ability enables stable production of seedless citrus fruit. Leigong No. 1 pummelo is an excellent local cultivar from Chenzhou, which is highly favored by consumers for its crisp flesh and distinctive flavor. However, this variety often presents a mixture of seedy and seedless fruit types in the market, which adversely affects its fresh consumption and industrial promotion. The under-

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lying cause of seedlessness in Leigong No. 1 pummelo remains unclear. Therefore, this study aims to investigate the mechanism of seedlessness in Leigong No. 1 pummelo, providing a theoretical basis for stable production of seedless fruits and for seedless breeding in citrus. **【Methods】** Mature flowers of Leigong No. 1 pummelo were dissected to examine floral organ morphology. Pollen viability was assessed using acetic carmine staining. Artificial emasculation was performed to evaluate the parthenocarpic ability of Leigong No. 1 pummelo. Self-pollination was conducted on Leigong No. 1 pummelo, followed by cross-pollination using it as the female parent and Cocktail grapefruit, Chunxiang tangelo, and Sanhong Miyou pummelo as male parents. At 5 days post-pollination, aniline blue staining was used to observe the pollen tubes from self-pollination and cross-pollination to determine whether fertilization was successful. The number of fruits and seeds was counted to calculate the fruit set rate and the number of seeds per fruit. Previously reported *S-RNase*-specific primers were used to identify the *S*-genotype of Leigong No. 1 pummelo, and semi-quantitative PCR was performed to analyze the expression pattern of *S-RNase* in this variety. **【Results】** Morphological examination of mature floral buds from Leigong No. 1 pummelo revealed normal floral structures, with both pistils and stamens well developed. Pollen viability assessed by acetic carmine staining was high across three consecutive years: 95.51% in 2022, 93.55% in 2023, and 96.50% in 2024, indicating male fertility. Artificial emasculation without pollination resulted in a fruit set rate of 14%, and all fruits were seedless, demonstrating that Leigong No. 1 pummelo exhibits a certain degree of parthenocarpy. Pollination experiments were performed to observe pollen tube growth 5 days after pollination. The results showed that, following self-pollination, pollen grains of Leigong No. 1 pummelo germinated abundantly on the stigma, but tube growth was arrested within the style, indicating self-incompatibility. In contrast, when Leigong No. 1 pummelo was used as the female parent and pollinated with Cocktail grapefruit, Chunxiang tangelo, or Sanhong Miyou pummelo, pollen tubes grew normally to the base of the style, demonstrating cross-compatibility and female fertility. Statistical analysis of fruit set and seed number in Leigong No. 1 pummelo showed that the self-pollination fruit set rate was 12.00%, with an average of 0.92 seeds per fruit. All 11 seeds obtained were shriveled and abnormal, and none were able to germinate into seedlings. In contrast, cross-pollination with Cocktail grapefruit, Chunxiang tangelo, or Sanhong Miyou pummelo resulted in fruit set rates of 8.00%, 43.94%, and 50.77%, respectively, with average seed numbers per fruit of 38.63, 45.72, and 75.52. The majority of these seeds were plump and viable, capable of developing into seedlings after germination and sowing. These results further confirm that seedlessness in Leigong No. 1 pummelo is primarily caused by self-incompatibility. Using published primers for citrus *S-RNase* genes, we identified the *S*-genotype of Leigong No. 1 pummelo as S_3S_5 . Semi-quantitative PCR revealed that both S_3 - and S_5 -*RNase* alleles were weakly expressed in petals, filaments, and anthers, but exhibited strong, tissue-specific expression in pistils, including the ovary, stigma, and style, with both showing higher relative expression levels in the style than in the ovary and stigma. **【Conclusion】** The seedless fruit formation of the local cultivar Leigong No. 1 pummelo from Chenzhou is mainly attributed to the combined effects of self-incompatibility and parthenocarpy. In production practices, implementing isolation planting to prevent cross-pollination can achieve stable production of seedless fruits. Furthermore, the self-incompatibility in this cultivar is a gametophytic type mediated by *S-RNase*, with the *S*-genotype identified as S_3S_5 . These results provide valuable reference data for the design of pollination combinations in future seedless citrus breeding programs, facilitating the development of improved citrus varieties with reliable seedless traits.

Key words: Leigong No. 1 pummelo; Seedlessness; Self-incompatibility; *S-RNase*

无核是柑橘果实的重要商品性状。无核柑橘食用方便,近年来已成为柑橘新品种选育的主要目标之一^[1]。多种育种途径可实现柑橘果实无核,如雄性不育、雌性不育、胚败育、自交不亲和、单性结实等^[2]。自交不亲和(self-incompatibility, SI)是植物抑制自交、促进异交的一种重要生殖机制,有助于防止近亲繁殖衰退并维持遗传多样性^[3],但也是果实无核的最主要原因^[4-6]。柚的自交不亲和抑制自身花粉进入子房,导致自身雌雄配子无法结合而造成果实无核,但若自交不亲和的柚被其他品种异花授粉,果实仍可产生种子。近年来报道的多个地方柚类品种,如橘湘红柚^[7]、东试早柚^[8]和贡水白柚^[9]等,其果实无核均由自交不亲和引起。尽管自交不亲和可产生无核果实,但也会因无法形成种子而影响坐果率。因此,稳定生产无核果实还需具备单性结实能力^[10]。如地方优良品种沙田柚和琯溪蜜柚均自交不亲和,但二者单性结实能力明显不同。沙田柚单性结实能力较差,在生产中往往需配置授粉树或进行人工授粉才能提高坐果率^[4];而琯溪蜜柚单性结实能力较强,不授粉即可稳定坐果而产生无核果实^[5]。

自交不亲和通常包括孢子体自交不亲和型(sporophytic self-incompatibility, SSI)和配子体自交不亲和型(gametophytic self-incompatibility, GSI)两种类型。在孢子体型自交不亲和植物中,柱头通过识别蛋白拒绝相同S基因型的花粉萌发^[11];而在配子体型自交不亲和植物中,尽管花粉能够在柱头萌发,但相同S基因型的花粉管在花柱中生长受阻,最终难以进入子房^[12]。前人研究表明,柑橘的自交不亲和属于S-RNase介导的配子体型自交不亲和,其中S-RNase为花柱特异性表达的雌性S决定因子^[13],而SLF(S-locus F-box)为花粉特异性表达的雄性S决定因子^[14]。S-RNase是一类核糖核酸糖蛋白,自花授粉时,花柱中S-RNase高表达,产生大量S-RNase进入花粉管,降解RNA导致花粉管生长停滞;而异花授粉时,花粉管中的多个SLF能够识别“非自我”的S-RNase,并将其降解,从而保障花粉管正常生长,穿过花柱进入子房以完成受精^[13, 15-16]。因此,S-RNase基因型鉴定对授粉配组具有重要指导价值。同时,研究柑橘S-RNase特异性表达,对进一步解析其配子体型自交不亲和分子机制具有重要意义。

郴州地方品种雷公1号柚果实肉质脆嫩、风味独特,深受消费者喜爱^[17],在郴州市临武县种植约

0.17万hm²,每666.7m²产量约2000kg。然而,其果实市场上存在有核与无核混杂现象,影响鲜食品质与产业发展。果实无核成因可能涉及花器官结构异常、花粉败育、雌性败育、自交不亲和等多种因素,但具体机制尚不清楚,且未见相关研究报道。鉴于此,笔者针对雷公1号柚上述产业问题,拟采取花器官形态观察、自花和异花授粉、花粉活力检测、花粉管生长观察、种子统计等方法来探究雷公1号柚果实无核成因,并在此基础上通过分子标记分析其无核机制,以期在无核化稳定生产及杂交育种提供理论依据。

1 材料和方法

1.1 材料

选择以枳为砧木的5年生雷公1号柚(*C. grandis* Osbeck)为材料,观察其花器官结构与花粉育性。随后,进行自交授粉,并以其为母本,与鸡尾葡萄柚(*C. paradisi* Osbeck)、春香橘柚(*C. tamurana* Tanaka × *C. natsudaidai* Hayata)和三红蜜柚(*C. grandis* Osbeck)3个父本进行杂交授粉。上述植物材料均定植在郴州市农业科学研究所果树基地。

1.2 花粉活力检测

授粉前,采用醋酸洋红染色法测定父本花粉活力。选取父本即将开放的成熟花朵,剥取花药制备花粉备用,花粉制备参考袁东亚^[18]的方法。使用棉签将花粉轻弹至涂有1%醋酸洋红染色液的载玻片上,染色2min后在显微镜下观察。具有活力的花粉,染色后呈现深红色或红色;败育或死亡花粉,染色后呈现浅粉红色或无色。随机统计约1000粒花粉染色情况并计算花粉活力,计算公式为:花粉活力/%=有活力的花粉数量/总花粉数量×100。

1.3 自交、杂交授粉与去雄套袋

以雷公1号柚为母本,采取去雄不授粉处理验证单性结实能力,以及自交与杂交授粉试验验证其自交亲和性。盛花期选取该品种健壮枝条上的花朵进行人工授粉,每个花序保留1~3朵即将开放的健壮花苞,将其余已开放或较小的花朵全部疏除。去雄不授粉处理方式:用镊子剥开花瓣去除雄蕊,尽量不要损伤雌蕊。自交与杂交(父本:鸡尾葡萄柚、春香橘柚、三红蜜柚)授粉处理方式:先用镊子将母本花中的雄蕊去除,随后用毛笔蘸取各父本花粉轻涂在柱头上。此外,自交与杂交授粉处理各额外授粉

10朵花,用于观察花粉管生长情况。最后,采用300目网袋对所有试验花朵进行套袋,并挂吊牌进行记录,5 d后拆除网袋。所有试验果实在成熟后采收,统计坐果率与种子数量。

1.4 自交与杂交授粉后的亲和性鉴定

授粉5 d后拆除网袋,采集上述额外授粉的10朵花,带回实验室,去除花瓣后,使用卡诺固定液(无水乙醇:冰醋酸=3:1)固定雌蕊(包含子房、花柱和柱头)24 h,经95%乙醇清洗3次后转至70%乙醇、4℃下保存备用。固定样品经蒸馏水漂洗2~3次,随后置于4 mol·L⁻¹ NaOH中,65℃水浴60 min使花柱软化,待其颜色由黄白色转为橙红色,弃去NaOH,使用蒸馏水漂洗花柱3~4次至透明黄色。0.1%苯胺蓝溶液染色花柱1~2 h后置于载玻片,滴加甘油和盖玻片压片,在正置荧光显微镜(Leica, DM6 B, Germany)下观察花粉管。

1.5 总DNA提取与S基因型鉴定

采用CTAB法提取雷公1号柚叶片DNA^[19]。参考已报道的21对*S-RNase*引物^[20]鉴定雷公1号柚S

基因型,引物(表1)由生工生物工程(上海)股份有限公司合成。PCR反应体系10 μL,各组分分为5.0 μL 2×Rapid Taq Master Mix,0.4 μL正引物F,0.4 μL反引物R,1.0 μL DNA,3.2 μL H₂O。PCR反应程序:95℃预变性3 min;95℃变性15 s,60℃退火15 s,72℃延伸15 s,30个循环;72℃终止延伸5 min,12℃保存备用。PCR扩增产物经1.0%琼脂糖凝胶电泳分离,使用凝胶成像系统(BIO-RAD, Universal Hood II, USA)观察电泳结果。

1.6 多组织cDNA提取与*S-RNase*基因表达模式分析

采集雷公1号柚的成熟花蕾,使用试剂盒(Hi-Pure HP Plant RNA Mini Kit, Magen)提取花瓣、花丝、花药、子房、花柱和柱头RNA,随后使用试剂盒(HiScript II Q RT SuperMix for qPCR (+gDNA wiper), Vazyme)逆转录RNA为cDNA。以各自cDNA和基因组DNA为模板,选择*Actin*^[21]作为内参基因(表1),使用*S-RNase*引物进行S基因表达模式分析。半定量PCR反应体系10 μL,各组分分为5.0 μL

表1 本研究中所用引物序列

Table 1 Primers sequence used in this study

引物名称 Primer name	引物序列(5'-3') Primer sequences		参考文献 Reference
<i>S</i> ₁	F: ATGAACATTACTTCTTCCTCTACA	R: GTTGATCTCCGTTCTTCGTG	[20]
<i>S</i> ₂	F: GACTAACCTCTTTTCGCTTTGC	R: CGGATCCATGCCTTTTCTAG	[20]
<i>S</i> ₃	F: TCCAACATCACCTATTGTACAGC	R: GATAACGACCATCACTAAAAACG	[20]
<i>S</i> ₄	F: GTTTGTTTCCTGCATTTCTC	R: GACCCCTGTTCTTTTTACTGC	[20]
<i>S</i> ₅	F: ATGAAGGTGGCATCCATCA	R: AATAATATCCGGCCACAG	[20]
<i>S</i> ₆	F: ATGGGGACTAATTTCTCATATCTTT	R: CTATATTTTAACGTATCGCGCAA	[20]
<i>S</i> ₇	F: ATCATCTCGGGCGCTGTTT	R: TCCCCACAGTTCTCGGTTTT	[20]
<i>S</i> ₈	F: CATTACCTATTCTGCTGCTCA	R: CCGAGTAGGGAACATGATTG	[20]
<i>S</i> ₉	F: GTTGTAACGCAAAACACTTCTG	R: CGTATGAGCATGTTAGTCTTGG	[20]
<i>S</i> ₁₀	F: CTGCATTGCCTCCGGCAC	R: TCGCCATTTTAACGGATCGAGG	[20]
<i>S</i> ₁₁	F: GCCGCTCCAACTCTTTTCG	R: CGCAGGGAACCTTGATGTTGT	[20]
<i>S</i> ₁₂	F: TGCTGCAATGGCCAACCTCTT	R: CCGGGTGGGGAACCTTGATG	[20]
<i>S</i> ₁₃	F: TCCACCACTTTTGGCTGGTC	R: TCTGCGCAACATTCGTCTCA	[20]
<i>S</i> ₁₄	F: AACGAAGGCAGCTTACCTCT	R: CACGGCAGCTCTCTCCATTA	[20]
<i>S</i> ₁₅	F: CAGCCTTGCTCGTTTGAAG	R: GCTTGATCGTCAGCGCAAAG	[20]
<i>S</i> ₁₆	F: CTACATCATAAATTTCTCCGGGC	R: GTGGTTTTGGTTCGTTTTCCC	[20]
<i>S</i> ₁₇	F: GCTCGGGGTTCAGAAAGT	R: ACGGTTTCAGGAAACTGATACG	[20]
<i>S</i> ₁₈	F: GCAGCTCAAACTCGTCGC	R: TAAACGGCAGGGGAGAGAA	[20]
<i>S</i> ₁₉	F: GCTCAAGCTGACCACTGTTG	R: CTACCAGCGTGGAGGAAAATA	[20]
<i>S</i> ₂₀	F: TCAGGTGCAGCAGAAAACAC	R: CTTGGCCGTCCACGCATATAA	[20]
<i>S</i> ₂₁	F: TCCACCACTTTTGGCTGGTC	R: CGGGTTTGGTTCGGGAAACT	[20]
<i>Actin</i>	F: ATCTGCTGGAAGGTGCTGAG	R: CCAAGCAGCATGAAGATCAA	[21]

2 × Rapid Taq Master Mix, 0.4 μL 正引物 F, 0.4 μL 反引物 R, 1.0 μL DNA, 3.2 μL H₂O。PCR 反应程序: 95 °C 预变性 3 min; 95 °C 变性 15 s, 58 °C 退火 15 s, 72 °C 延伸 15 s, 30 个循环; 72 °C 终止延伸 5 min, 12 °C 保存。PCR 扩增产物经 1.0% 琼脂糖凝胶电泳分离, 使用凝胶成像系统 (BIO-RAD, Unversal Hood II, USA) 观察电泳结果。

2 结果与分析

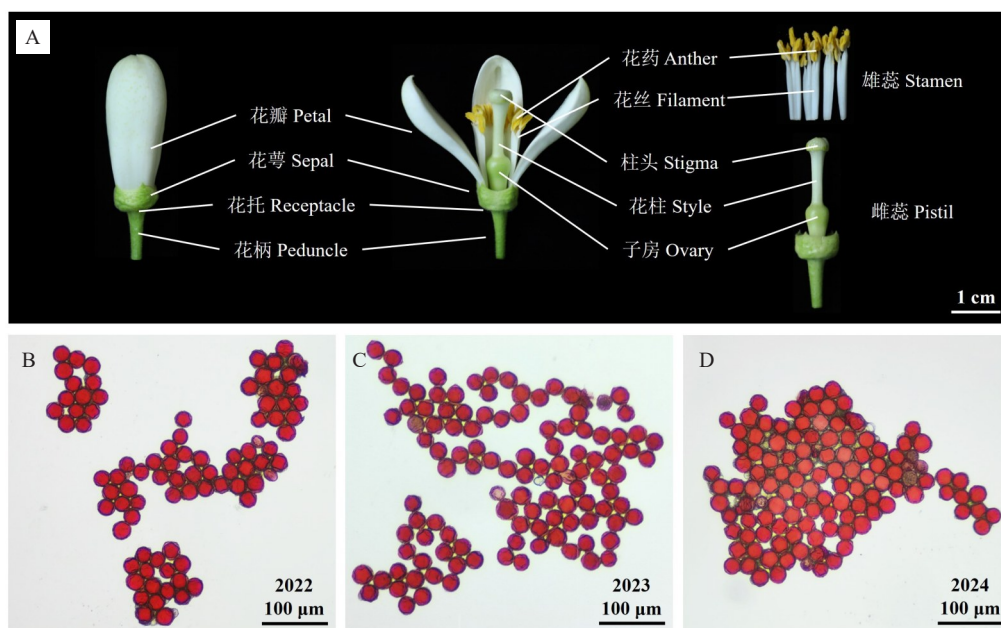
2.1 雷公1号柚花器官形态观察与花粉活力检测

随机选择 10 朵雷公 1 号柚即将开放的成熟花苞, 观察其花器官结构 (图 1)。结果显示, 该品种无

明显柱头外露、柱头退化、花柱细长等异常现象, 柱头上存在较多黏液, 子房外观正常; 花药发育饱满, 散粉后表现为橙黄色, 与多数柑橘正常花药、花粉表型一致。随后, 2022—2024 年连续 3 年雷公 1 号柚花粉活力染色观察显示, 2022 年为 95.51%、2023 年为 93.55%、2024 年为 96.50%, 表明该品种具有较高的花粉活力。综上所述, 雷公 1 号柚花器官外观正常完整, 属于完全花, 且花粉活力较高, 表明其雄性可育。

2.2 雷公1号柚自交与杂交亲和性鉴定

为分析雷公 1 号柚的自交亲和性, 以该品种为母本, 自交授粉 5 d 后, 观察到大量花粉在柱头上萌发, 表明自身花粉正常可育, 但花粉管在花柱中生长



A. 花器官; B-D. 2022—2024 年花粉染色。

A. The flower organ; B-D. Pollen staining from 2022—2024.

图 1 雷公 1 号柚花器官结构与花粉醋酸洋红染色

Fig. 1 The floral organ structure and acetocarmine staining pollen of Leigong No.1 pummelo

受阻, 难以到达花柱底部, 表现出自交授粉不亲和的特征 (图 2-A)。进一步分析雷公 1 号柚的异交亲和性, 以雷公 1 号柚为母本, 与鸡尾葡萄柚、春香橘柚、三红蜜柚为父本进行杂交, 杂交授粉 5 d 后, 观察到这 3 个父本品种的花粉在柱头上能够大量萌发, 且花粉管均顺利生长至花柱底部, 表现出杂交授粉亲和的特征 (图 2-B~D)。以上结果表明, 雷公 1 号柚果实无核可能是由自交不亲和导致。

2.3 雷公1号柚去雄与授粉的坐果率与种子观察

以雷公 1 号柚为母本, 开展去雄不授粉、自交授

粉和杂交授粉试验, 以验证该品种的单性结实能力和授粉亲和性 (图 3, 表 2)。结果表明, 雷公 1 号柚去雄不授粉试验坐果率为 14.00%, 果实均无核, 表明该品种具备一定的单性结实能力; 自交授粉试验坐果率为 12.00%, 单果种子数很少, 几乎无核, 仅 0.92 粒·果⁻¹, 且产生的 11 粒种子普遍干瘪偏小 (图 3-F), 通过催芽播种未能萌发, 表明自交难以产生正常可育的种子。杂交授粉试验中, 雷公 1 号柚 × 鸡尾葡萄柚、雷公 1 号柚 × 春香橘柚、雷公 1 号柚 × 三红蜜柚 3 个组合的坐果率分别为 8.00%、43.94% 和

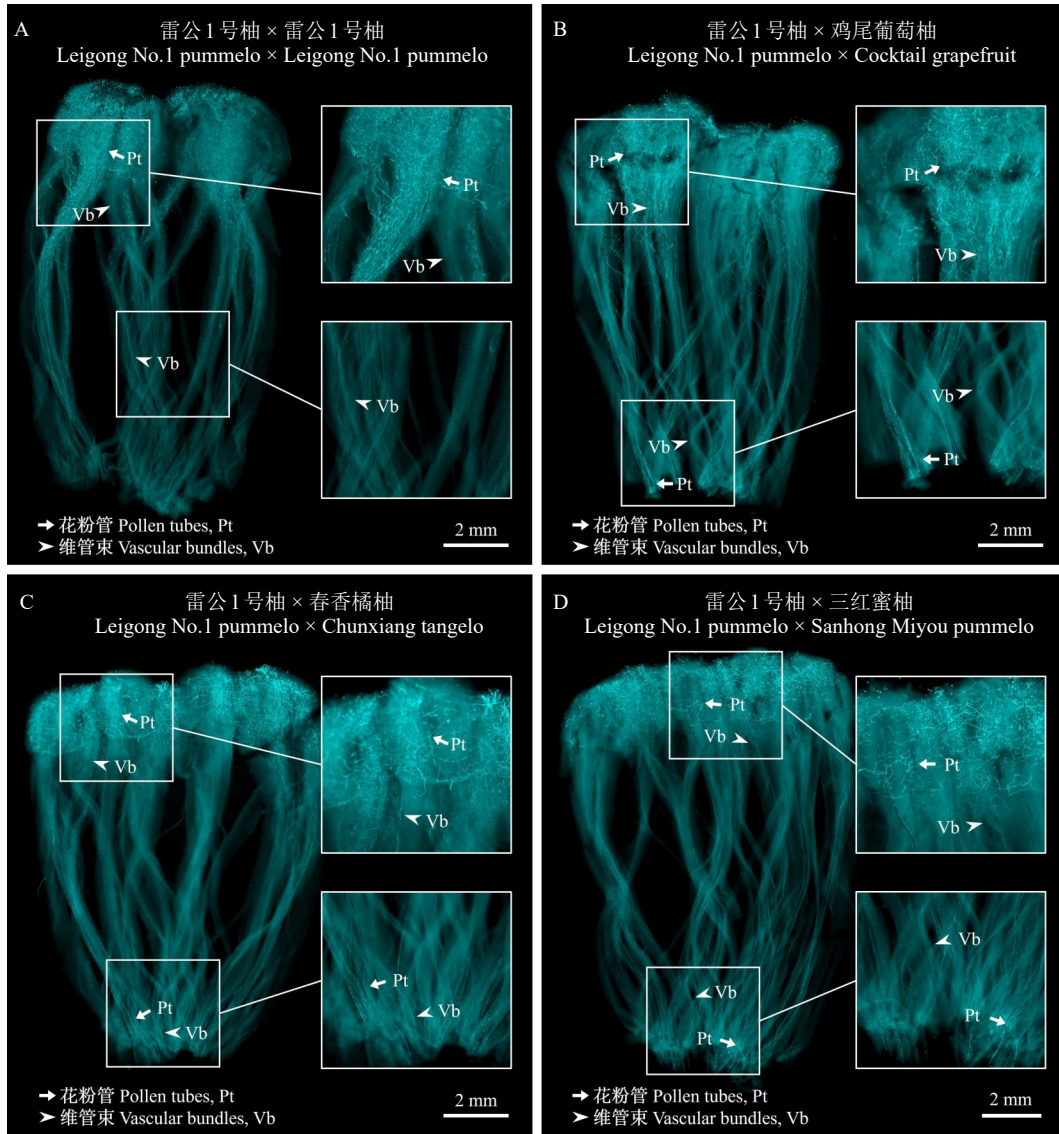


图 2 雷公 1 号柚授粉 5 d 后花柱苯胺蓝染色

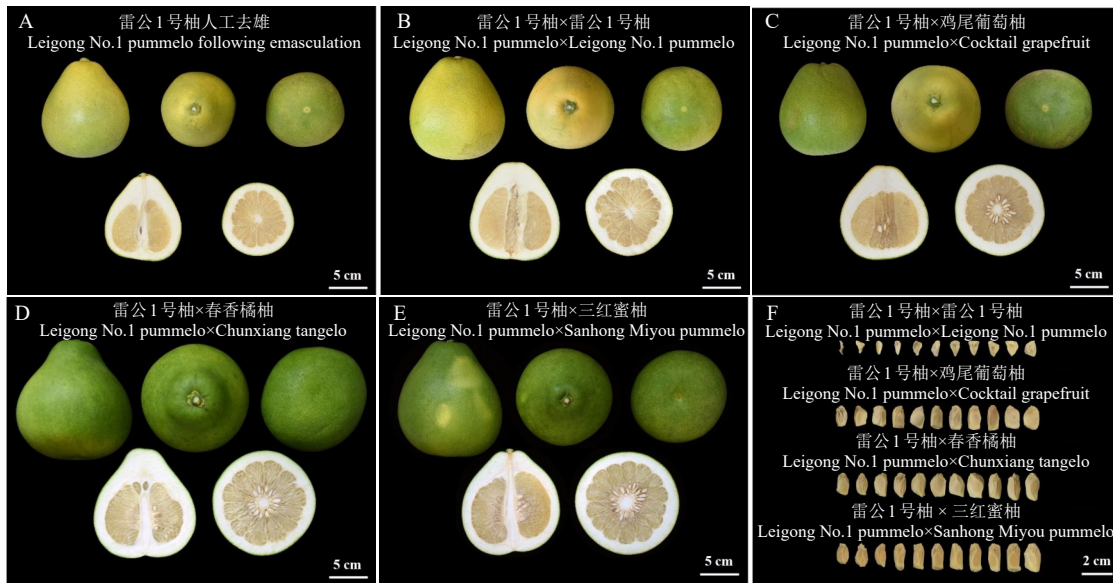
Fig. 2 The style of Leigong No.1 pummelo was stained with aniline blue after 5 days of pollination

50.77%,单果种子数分别为 38.63、45.72、75.52 粒·果⁻¹,均产生较多饱满种子(图 3-F),表明雷公 1 号柚胚囊发育正常,通过杂交授粉能产生正常种子。综上所述,雷公 1 号柚胚囊发育正常,能够产生可育雌配子,表现出自交授粉不亲和与杂交授粉亲和的特点,且具备一定的单性结实能力。推测雷公 1 号柚部分果实产生种子可能与周边其他品种发生异交有关。

2.4 雷公 1 号柚 *S* 基因型鉴定与 *S-RNase* 基因表达分析

提取雷公 1 号柚叶片 DNA,使用已报道的 21 对 *S-RNase* 引物对其进行 *S-RNase* 基因扩增,分析 *S-RNase* 基因型。PCR 扩增结果显示,该品种在 *S*₃

与 *S*₅ 引物下扩增出特异性条带(图 4-A),表明该品种 *S* 基因型为 *S*₃*S*₅。随后,提取花瓣、花丝、花药、子房、花柱和柱头 RNA,逆转录为各自 cDNA,采取半定量 PCR 分析 *S*₃ 与 *S*₅ 基因表达模式。结果显示, *S*₃ 与 *S*₅ 在该品种花瓣、花丝和花药中均表达不明显,但均在雌蕊(子房、柱头和花柱)中表达量较高,证明 *S*₃ 与 *S*₅ 的表达存在组织特异性(图 4-B~C)。此外,花柱上 *S*₃ 与 *S*₅ 扩增条带的荧光强度,明显高于柱头与子房,表明 *S*₃ 与 *S*₅ 在该组织上的相对表达水平高于子房与柱头。以上数据表明,雷公 1 号柚 *S-RNase* 基因型为 *S*₃*S*₅, *S*₃ 与 *S*₅ 均在雌蕊(子房、柱头和花柱)中特异性表达,且花柱中的相对表达水平最高。



A. 去雄; B. 自交; C-E. 杂交; F. 各处理下种子表现。

A. Emasculating; B. Selfing; C-E. Crossing; F. Seed performance of each treatment.

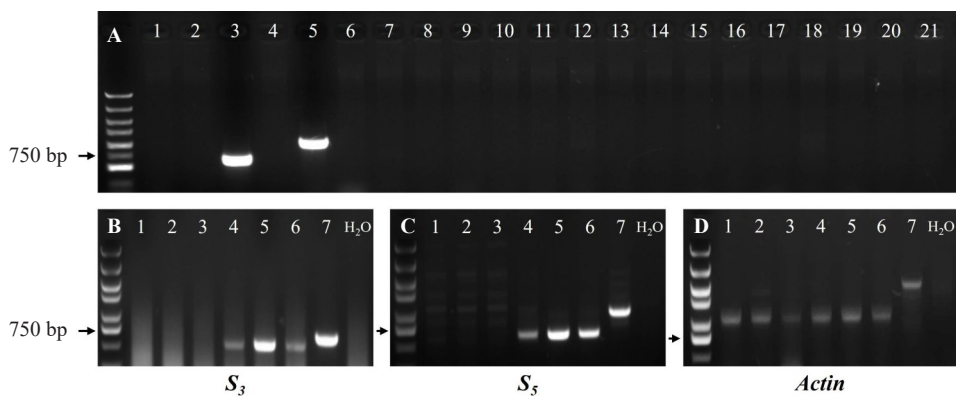
图 3 雷公 1 号柚处理后果实与种子发育情况

Fig. 3 Fruit and seed development of Leigong No.1 pummelo after treatment

表 2 雷公 1 号柚坐果率及单果种子数目统计

Table 2 Fruit setting rate and seed number of Leigong No.1 pummelo

年度 Year	组合 Combination	处理花朵数 No. of treated flower	坐果数 No. of fruit	坐果率 Fruit setting rate/%	种子数 No. of seed	单果种子数 No. of seed per fruit
2022	雷公 1 号柚人工去雄 Leigong No.1 pummelo following emasculation	100	14	14.00	0	0.00
2022	雷公 1 号柚 × 雷公 1 号柚 Leigong No.1 pummelo × Leigong No.1 pummelo	100	12	12.00	11	0.92
2022	雷公 1 号柚 × 鸡尾葡萄柚 Leigong No.1 pummelo × Cocktail grapefruit	100	8	8.00	309	38.63
2023	雷公 1 号柚 × 春香橘柚 Leigong No.1 pummelo × Chunxiang tangelo	66	29	43.94	1326	45.72
2024	雷公 1 号柚 × 三红蜜柚 Leigong No.1 pummelo × Sanhong Miyou pummelo	65	33	50.77	2492	75.52



A. *S* 基因型鉴定(1~21: S_1 - S_{21}); B. S_3 基因表达模式; C. S_5 基因表达模式; D. *Actin* 基因表达模式(1. 花瓣; 2. 花丝; 3. 花药; 4. 子房; 5. 花柱; 6. 柱头; 7. DNA)。

A. *S*-genotypes identification (1-21: S_1 - S_{21}); B. Expression pattern of S_3 -Rnase; C. Expression pattern of S_5 -Rnase; D. Expression pattern of the *Actin* gene (1. Petal, 2. Filament, 3. Anther, 4. Ovary, 5. Style, 6. Stigma, 7. DNA).

图 4 雷公 1 号柚 *S* 基因型鉴定与 *S-RNase* 基因表达模式分析

Fig. 4 *S*-genotypes identified and expression pattern analysis of *S-RNase* in Leigong No.1 pummelo

3 讨 论

研究表明,多种途径可导致柑橘无核,包括雄性不育、胚囊异常、胚败育、自交不亲和、单性结实等。雄性不育是常见的无核成因,例如胞质杂种华柚3号因雄蕊退化,花药发育不全、花粉败育,隔离种植下果实完全无核^[22]。雷公1号柚雌雄蕊器官发育正常,花粉活力连续3年均超过90%。该品种自交花粉可在柱头上萌发,但花粉管在花柱中生长受阻;而杂交花粉管则能顺利到达花柱底端,表现出配子体型自交不亲和特性。同时,自交种子数远低于杂交授粉的种子数,且产生的11粒自交种子均为瘪籽,而杂交种子则多为饱满籽,表明雷公1号柚果实无核现象主要源于自交不亲和,而非雄性不育、胚囊异常或胚败育所致。这一结果与Liang等^[13]关于394份柚类自交不亲和普遍存在的结论一致。尽管自交不亲和可导致授粉失败,但无核果实的形成仍需依赖单性结实能力。雷公1号柚去雄坐果率14.00%,高于沙田柚(1.95%)、琯溪蜜柚(5.43%)^[23]和贡水白柚(9%)^[9]。Zhou等^[24]对50个柚品种进行去雄处理,花后1个月统计坐果率,其中22个品种为1.8%~28.0%,其余28个品种为0,说明雷公1号柚的单性结实能力在柚类中属于较高水平。温州蜜柑的单性结实能力可遗传^[24],挖掘强单性结实柑橘种质对无核育种具有重要意义。综上所述,雷公1号柚属于配子体型自交不亲和,其无核果实的形成主要由自交不亲和与单性结实共同作用所致,隔离种植下可稳定生产无核果实。未来可深入研究其单性结实机制,并通过与弱单性结实品种杂交创制新种质。

笔者在本研究中成功鉴定出雷公1号柚的S基因型为 S_3S_3 ,并证实 S_3 与 S_3 均在雌蕊组织(柱头、花柱和子房)中特异性表达,且均在花柱上的相对表达水平最高。柑橘种子的形成依赖于授粉受精的成功,自交不亲和可导致授粉受精障碍而影响种子形成。柑橘属于*S-RNase*介导的配子体型自交不亲和^[13],不同柑橘品种通常具备不同的S基因型^[6]。雷公1号柚表现出自交不亲和特性,其S基因型被鉴定为 S_3S_3 ,而文献报道三红蜜柚为 S_3S_3 ^[5]。由于两者S基因型不同,理论上通过杂交能够克服授粉不亲和障碍而产生正常种子。为验证上述推测,开展授粉与半定量PCR试验。结果显示,相同S基因型间授粉(雷公1号柚×雷公1号柚),花粉管在花柱中生长受阻,

仅形成极少量异常种子且无法培育成苗;不同S基因型间授粉(雷公1号柚×三红蜜柚),花粉管能生长至花柱底端,最终形成大量饱满种子,经过催芽播种可培育成苗。此外,花期检测到 S_3 与 S_3 均在雷公1号柚的花柱中高表达,表明花柱可能是抑制花粉管生长的重要位置。而前人研究表明,不同品种的花粉管并非都在花柱中停止生长,如高班柚自交花粉管在柱头内受阻,在靠近花柱顶端处停止生长^[5];无籽砂糖橘自交花粉管可到达花柱底部,但在子房附近停止生长^[25],说明*S-RNase*基因在雌蕊的3个组织中表达,可能是作为抑制相同*S-RNase*基因花粉管生长的三道生理屏障,从而影响授粉亲和性。试验结果与理论推测一致,充分证明雷公1号柚属于*S-RNase*介导的配子体型自交不亲和。同时,明确S基因型对柑橘授粉配组具有重要指导意义,以及*S-RNase*基因的特异性表达对介导花粉管生长具有关键作用。

4 结 论

郴州地方优良品种雷公1号柚果实无核主要由自交不亲和与单性结实共同导致,在生产上采取隔离种植可实现无核果实稳定生产。同时,该品种自交不亲和属于*S-RNase*介导的配子体型自交不亲和,其*S-RNase*基因型被鉴定为 S_3S_3 , S_3 与 S_3 均在雌蕊组织中特异性表达,且在花柱上相对表达水平最高,该结果能为后续无核育种的授粉配组和S基因研究提供理论依据。

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